

**Amendments to the Specification:**

A. Please replace the fifth full paragraph on page 5, with the following amended paragraph:

**FIGS. 5A to 5AT 5.1 to 5.46** present the coordinates for the crystal structure of Aequorea-related green fluorescent protein S65T **(SEQ ID NOS:5,6)**.

B. Please replace Table B which starts at line 24 on page 30, with the following amended table:

TABLE B

	Original position and presumed role	Change to	Codon
L42	Aliphatic residue near C=N of chromophore	<u>CFHLQRWYZ (SEQ ID NO:7)</u>	5'YDS 3' 3'RHS 5'
V61	Aliphatic residue near central —CH=— of chromophore	<u>FYHCLR (SEQ ID NO:8)</u>	YDC RHg
T62	Almost directly above center of chromophore bridge	<u>AVFS (SEQ ID NO:9)</u> <u>DEHKNO (SEQ ID NO:10)</u> <u>FYHCLR (SEQ ID NO:8)</u>	KYC MRg VAS BTS YDC RHg
V68	Aliphatic residue near carbonyl and G67	<u>FYHL (SEQ ID NO:11)</u>	YWC RWg
N121	Near C—N site of ring closure between T65 and G67	<u>CFHLQRWYZ (SEQ ID NO:7)</u>	YDS RHS
Y145	Packs near tyrosine ring of chromophore	<u>WCFL (SEQ ID NO:12)</u> <u>DEHNKQ (SEQ ID NO:13)</u>	TKS AMS VAS BTS
H148	H-bonds to phenolate oxygen	<u>FYNI (SEQ ID NO:14)</u> <u>KQR</u>	WWC WWg MRg KYC
V150	Aliphatic residue near tyrosine ring of chromophore	<u>FYHL (SEQ ID NO:11)</u>	YWC RWg
F165	Packs near tyrosine ring	<u>CHQRWYZ (SEQ ID NO:15)</u>	YRS RYS
I167	Aliphatic residue near phenolate; I167T has effects	<u>FYHL (SEQ ID NO:11)</u>	YWC RWg
T203	H-bonds to phenolic oxygen of chromophore	<u>FHLQRWYZ (SEQ ID NO:16)</u>	YDS RHS
E222	Protonation regulates ionization of chromophore	<u>HKNO (SEQ ID NO:17)</u>	MAS KTS

C. Please replace Table C which starts at line 1 page 32 with the following amended table:

TABLE C

	Original position and presumed role	Change to	Codon
Q69	Terminates chain of H-bonding waters	<u>KREG (SEQ ID NO:18)</u>	RRg YYC
Q94	H-bonds to carbonyl terminus of chromophore	<u>DEHKNO (SEQ ID NO:10)</u>	VAS BTS
Q183	Bridges Arg96 and center of chromophore bridge	<u>HY</u> <u>EK</u>	YAC RTG RAg YTC
N185	Part of H-bond network near carbonyl of chromophore	<u>DEHNKQ (SEQ ID NO:13)</u>	VAS BTS

D. Please replace Table D, which starts at line 26, page 32, with the following amended table:

TABLE D

	Original position and presumed role	Change to	Codon
L220	Packs next to Glu222; to make GFP pH sensitive	<u>HKNPQT (SEQ ID NO:19)</u>	MMS KKS
V224	Packs next to Glu222; to make GFP pH sensitive	<u>HKNPQT (SEQ ID NO:19)</u> <u>CFHLQRWYZ (SEQ ID NO:7)</u>	MMS KKS  YDS RHS

E. Please replace paragraph the bridging pages 44 and 45 with the following amended paragraph:

[0161] The invention can also include a localization sequence, such as a nuclear localization sequence, an endoplasmic reticulum localization sequence, a peroxisome localization sequence, a mitochondrial localization sequence, or a localized protein. Localization sequences can be targeting sequences which are described, for example, in "Protein Targeting", chapter 35 of Streyer, L., *Biochemistry* (4th ed.). W. H. Freeman, 1995. The localization sequence can also be a localized protein. Some important localization sequences include those targeting the nucleus (KKKRK, SEQ ID NO:20), mitochondrion (amino terminal MLRTSSLFTR-RVQPSLFRNILRLQST-, SEQ ID NO:21), endoplasmic reticulum (KDEL, SEQ ID NO:22, at

C-terminus, assuming a signal sequence present at N-terminus), peroxisome (SKF at C-terminus), prenylation or insertion into plasma membrane (CaaX, CC, CXC, or CCXX at C-terminus), cytoplasmic side of plasma membrane (fusion to SNAP-25), or the Golgi apparatus (fusion to furin).

F. Please replace the paragraph bridging pages 60 and 61 with the following amended paragraph:

**[0208]** Although the electron density map is for the most part consistent with the proposed structure of the chromophore (D. C. Prasher et al. *Gene* 111:229-233 (1992); C. W. Cody et al. *Biochemistry* 32:1212-1218 (1993)) in the *cis* [Z-] configuration, with no evidence for any substantial fraction of the opposite isomer around the chromophore double bond, difference features are found at  $>4 \sigma$  in the final ( $F_o$ - $F_c$ ) electron density map that can be interpreted to represent either the intact, uncyclized polypeptide or a carbinolamine (inset to **FIG. 2C**). This suggests that a significant fraction, perhaps as much as 30% of the molecules in the crystal, have failed to undergo the final dehydration reaction. Confirmation of incomplete dehydration comes from electrospray mass spectrometry, which consistently shows that the average masses of both wild-type and S65T GFP ( $31,086 \pm 4$  and  $31,099.5 \pm 4$  Da, respectively) are 6-7 Da higher than predicted (31,079 and 31,093 Da, respectively) for the fully matured proteins. Such a discrepancy could be explained by a 30-35% mole fraction of apoprotein or carbinolamine with 18 or 20 Da higher molecular. The natural abundance of  $^{13}\text{C}$  and  $^2\text{H}$  and the finite resolution of the Hewlett-Packard 5989B electrospray mass spectrometer used to make these measurements do not permit the individual peaks to be resolved, but instead yields an average mass peak with a full width at half maximum of approximately 15 Da. The molecular weights shown include the His-tag, which has the sequence MRGSHHHHHH GMASMTGGQQQM GRDLYYDDDDDK DPPAEF (SEQ ID NO:5-NO:23). Mutants of GFP that increase the efficiency of fluorophore maturation might yield somewhat brighter preparations. In a model for the apoprotein, the Thr<sup>65</sup>-Tyr<sup>66</sup> peptide bond is approximately in the  $\alpha$ -helical conformation, while the peptide of Tyr<sup>66</sup>-Gly<sup>67</sup> appears to be tipped almost perpendicular to the helix axis by its interaction with Arg<sup>96</sup>. This further supports the speculation that Arg<sup>96</sup> is important in generating the conformation

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required for cyclization, and possibly also for promoting the attack of Gly<sup>67</sup> on the carbonyl carbon of Thr<sup>65</sup> (A. B. Cubitt et al. *Trends Biochem. Sci.* 20:448-455 (1995)).